WS16.1 Development of a competitive ELISA for the detection of the *Burkholderia cenocepacia* siderophore, ornibactin

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The acquisition of iron is critical for the survival of pathogenic bacteria including the cystic fibrosis (CF) pathogen *B. cenocepacia.* Siderophores are low molecular mass iron chelators secreted by bacteria in order to solubilise and internalise ferric iron. Ornibactin is the most abundant of four siderophores produced by *B. cenocepacia* and is pivotal to the virulence of this opportunistic pathogen. An accurate method of quantifying this important virulence factor will facilitate both pathogenesis studies and the development and evaluation of therapies targeting iron acquisition. Ornibactin was extracted and purified from *B. cenocepacia* iron-depleted cultures and identified using reverse-phase HPLC and LC-MS analysis. Purified ornibactin was then conjugated to the carrier protein BSA using photo-activation chemistry. Resultant polyclonal antibodies against the ornibactin-conjugate were raised in rabbits and the presence of serum antibodies to both ornibactin and ferric-ornibactin confirmed using immunoblot and Western blot analysis. A competitive ferric-ornibactin ELISA was then developed by coating ELISA plates with a second ornibactin-BSA conjugate to which the positive rabbit sera and ornibactin positive sample compete for binding. Bound antibodies are detected using an anti-rabbit HRP conjugate which catalyses a colorimetric reaction. The linear range of the ELISA occurs between 0 and 500 ng/ml indicating an extremely sensitive assay. The ferric-ornibactin competitive ELISA is currently under evaluation for the detection of ferric-ornibactin in a range of clinical specimens.

WS16.2 Highly multiplexed molecular detection of CF lung infections

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Objectives: Culture-based detection of micro-organisms causing CF lung infection is slow, labour intensive and may overlook fastidious or slow-growing pathogens. This may hinder prompt and directed antibiotic treatment of pulmonary exacerbations. We aimed to develop a highly multiplexed molecular diagnostic assay for the simultaneous detection of bacteria, fungi and viruses directly from sputum without prior culture.
Methods: Unique primer and probe sets compatible with an existing biochip array platform (Evidence Investigator, Randox Laboratories Ltd) were designed to detect 120 species common to CF lung infections and to identify them to the species or genus level as judged clinically relevant. Primers and probes were tested for sensitivity and specificity in detecting multiple target isolates before trialling with clinical sputum samples. Real-time PCR assays, culture data and next generation sequencing were used as comparator technologies.
Results: Target specificities and sensitivities were confirmed for all primer designs and a high number of primer/probe sets were successfully trialled with sputum samples. Sensitivities and specificities of 90–100% and 99–100%, respectively, were achieved with clinical samples.
Conclusion: Based on multiplex PCR coupled to biochip array technology, we have developed a unique multiplex molecular diagnostic assay tailored towards the direct detection and identification of a comprehensive profile of agents contributing to CF lung infection. Specificities and sensitivities indicate that the assay format is highly suitable for this application and full clinical trialling is planned.
Funding: KTP between QUB, Randox Laboratories Ltd and TSB.

WS16.3 Rethinking *Pseudomonas aeruginosa* (PA) lung infection: using molecular microbiology rather than culture and antibodies

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Objective: We have revisited the adopted definition of the initial and persistent *P. aeruginosa* (PA) lung infection in CF patients by combining genomics, molecular microbiology and clinical data.
Methods: Clinical isolates of PA have been systematically collected for 10 years from early infection stages in Copenhagen CF children and young adults. Based on clinical characterization more than 500 isolates from 41 patients were chosen for genome sequencing.
Results: More than 75% of all CF patients were infected with the same clone-type during the entire collection period, despite intensive antibiotic therapy and although the bacteria were eradicated from the sputum at the next microbiology examination. Four types of infection patterns dominated 1) same persistent clone type, 2) same persistent clone type with single invaders, 3) several co-existing clone types or 4) clone replacement.
Conclusion: Today the definition of intermittent or chronic PA lung infection is based on clinical and laboratory findings, e.g. intermittent colonisation: PA in $<50\%$ of sputum samples or chronic infection: PA in $\geqslant 50\%$ of sputum samples within one year, respectively. In addition increasing ELISA or precipitating antibodies against PA is a predictor of infection in some centres. Molecular methods, however, show that the majority of patients are infected persistently with the same clone type from the very beginning. Most CF patients are therefore either non-colonised or chronically infected; this needs to be reflected in the way the initial PA colonisation is treated, which, based on molecular evidence, should be treated intravenously right from the start and for prolonged periods.

WS16.4 Serum-betaglucan as marker to differentiate between fungal airway colonization and infection in patients with cystic fibrosis

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Objectives: Growth of fungi in cultures from airway secretions of CF patients are difficult to interpret since this can represent both colonisation and infection. Betaglucan is an highly fungi-specific antigen, an elevated serum-concentration of betaglucan (S-BG) is usually interpreted as sign for invasive fungal infection. To investigate if S-BG can differentiate between fungal colonisation and infection in CF patients.
Methods: S-BG, SR and CRP were analysed at the annual review. All airway cultures during the last year, presence of ABPA and treatment with antimycotics were reviewed.
Results: Until now, we analysed S-BG in 37 patients (1–17 yrs). S-BG was low (<60 pg/ml) in 23 patients (group 1, mean age 8.0 yrs), intermediate (60–80 pg/ml) in 5 (group 2, mean age 8.6 yrs) and pos (>80 pg/ml) in 9 (group 3, mean age 10.5 yrs). No patient had signs of ongoing invasive fungal infection. In group 1, none had growth of fungi in >50% of all cultures but 2 patients in group 2 (*Exophiala*, *Aspergillus*) and 3 patients in group 3 (yeast, *Exophiala*). One patient in group 1 was treated with antimycotics, 1 in group 2 and 3 in group 3. There was one patient with ABPA in group 2 and one in group 3. CRP and SR were normal in all except 2 patients. Mean FEV1% was 90% in group 1, 83% in group 2 and 81% in group 3.
Conclusion: Patients with elevated S-BG were older, more often on antimycotics, had lower FEV1% and more positive cultures. An elevated S-BG did not automatically indicate infection and a low S-BG did not rule out infection. This indicates that S-BG only can function as a complementary marker to differentiate between colonization and infection. The study is ongoing.